Submission date	30/09/2022
Each year deadline	September 30

Acronym of the R&T project (max 10 characters)

TRAINBYCLEM

Title of the R&T project (max 100 characters including spaces)

Bridging molecular and structural data to determine the identity of IFT trains by correlative microscopy

Summary of the R&T project (max 500 characters including spaces)

Flagella assemble by addition of new subunits at the distal end. Proteins are delivered by the movement of protein complexes (or trains). We have visualised train movement and established their 3D distribution. However, the connection between molecular and structural identity remains a challenge. In this collaborative project, we will develop advanced correlative light and electron microscopy approaches to bridge that gap, using wild-type and mutant cells where train construction is defective.

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R&T project (max 2,500 characters including spaces)

Cilia and flagella are essential organelles in most eukaryotic cells. They are characterised by a structure of 9 doublet microtubules called axoneme and composed of hundreds of proteins whose assembly takes place via addition of new subunits at the distal end. Protein delivery is

ensured by intraflagellar transport (IFT), the movement of protein complexes (or trains) taking place between microtubules and the flagellar membrane.

Trains are protein polymers that carry flagellar components and are moved by kinesin and dynein motors. We investigate IFT in the protist *Trypanosoma brucei*, a great model organism for imaging and reverse genetics. It branched early and provides clues in terms of cilia evolution (Mallet & Bastin, 2022). Our teams have visualised train movement using light microscopy and examined their ultrastructural distribution with electron microscopy (Bertiaux et al. 2018). Our data reveal that, surprisingly, IFT trains are using only 4 out of 9 doublets for trafficking. We next moved to high resolution imaging to show that anterograde and retrograde IFT were taking place on both sides of the axoneme. This could be explained by least three models (see Figure).

To find out which one is correct, we will establish the connection between molecular and structural data by a correlative light and electron microscopy approach (CLEM). In this collaborative project between the TRYPA and UBI Units, we will monitor IFT thanks to cell lines expressing IFT fluorescent proteins. Trypanosomes will be immobilised on slides and IFT will be recorded. Rapid fixation (<200 ms) will allow the positioning of each fluorescent train and the sample will then be analysed by transmission electron microscopy. This will reveal the molecular identity of each train, its structure and its exact position. First studies will be performed in a wild-type background, and next in our collection of cells lines where distinct components of the IFT machinery have been depleted.

This will rely on advanced CLEM, as only performed so far in the green alga *Chlamydomonas* (Stepanek & Pigino, 2016). It will answer the question about the precise IFT train positioning in trypanosomes and will provide the first comparison with another organism, hence bringing new evolutionary insights in IFT evolution and its relationship with the shaping of different types of cilia and flagella (Mallet & Bastin, 2022), a critical aspect to understand how the different types of cilia of the human body are assembled.

R&T added value of the co-direction of this project (max 1,000 characters including spaces)

This project relies on the complementary expertise of UBI providing technological development and TRYPA expertise in IFT. The project will answer a central question in the field and will foster interactions between the two teams, in a win-win configuration. To answer the biological questions detailed in the scientific project, a methodological. The project focuses on the study of intraflagellar transport in Trypanosoma brucei. Two axes are developed: the first is to precisely define the location of IFT complexes on the doublets of microtubules that make up the axoneme and the second is to understand the mode of assembly of IFT trains. These two lines of research are based on a central technology, electron microscopy. To answer the biological questions detailed in the scientific project, a methodological development is to be implemented.
This co-direction will allow the student to acquire a **dual scientific culture** including research and technological development. The student will be able to acquire cutting-edge expertise on intraflagellar transport in **cell biology and electron microscopy** thanks to the specific skills of each co-supervisor. The biology part of the project will be under the direction of Philippe

Bastin (Thesis director) and the methodological implementation part will be under the direction of Adeline Mallet (co-director).
The student will benefit from an exceptional work environment including access to the latest generation equipment and close interactions with technological experts. The student will be integrated into the two units, will participate in the respective team meetings and in the seminars of the fields of expertise (for example: cilia meeting, focus on Microscopy).
This project will also open doors to careers in technological research for students.

Organisation of the project among partners (work packages, roles and communication; max 1 page)

Year 1
WP1: set up appropriate conditions for imaging IFT and for rapid fixation. Preliminary experiments revealed that it was possible to visualise and record IFT in cells expressing the IFT172 protein fused to TandemTomato, and to fix almost instantly (~200 ms) by addition of glutaraldehyde. The sample is then ready to be processed for electron microscopy. The movement of IFT trains is easily determined thanks to kymograph analysis.

WP2: set up optimal conditions for electron microscopy analysis. Samples with long portions of flagella in focus will be investigated first and thick sections will be used for tomography analysis. The aim is to visualise a large number of IFT trains at high resolution.

Year 2
WP3: position signals emitted by IFT trains on electron microscopy pictures to correlate light and electron microscopy data. Thanks to the information provided by the kymograph, the directionality of the moving trains is known and its structure can now be identified thanks to the electron microscopy pictures. This will solve the longstanding question of the identity of IFT trains first identified more than 15 years ago.

WP4: perform quantitative data analysis to establish the structure of anterograde and retrograde trains, as well as their positioning along the axoneme.

Year 3
WP5: reproduce the analysis on cell lines where assembly or trafficking of IFT trains has been perturbed by RNAi knockdown of key components. This will reveal the role of motors (kinesin, dynein) and of the two family of IFT proteins. These cell lines have been developed by the TRYPA lab and contain various fluorescent markers as IFT reporters.

WP6: paper and thesis writing up

The candidate will work in both units, performing the live imaging in the TRYPA unit (with support of the PBI facility) and the electron microscopy at UBI. As said above, the two teams have worked together for many years and are used to meet on a regular basis. In this specific context, monthly meetings will be organised between the future student and the two supervisors. The student will participate in the lab meetings of the two units. Other UBI or

TRYPA members will be involved for training in trypanosome cell culture and imaging and for electron microscopy.

The student will also participate to at least one international conference and one national conference every year. These will be selected based on the calendar, with the aim to alternate between cell biology and electron microscopy.

R&T candidate background required for developing the project

After a Master degree, the candidate must have a solid knowledge in cell biology and good notions of microscopy.
The person selected for this project will have to demonstrate versatility and ease of communication to work in multidisciplinary teams. Obviously, the candidate must have a significant motivation for challenging questions and the development of new methods.

Selected publications or patents (max 5) of partners

1. Buisson, J., N. Chenouard, T. Lagache, T. Blisnick, J.C. Olivo-Marin, and **P. Bastin**. 2013. Intraflagellar transport proteins cycle between the flagellum and its base. J Cell Sci. 126:327-338. First demonstration of IFT recycling in any organism. *Highlighted by the Journal. 2nd highest download of the journal.*

2. Bertiaux, E.*, **A. Mallet***, C. Fort, T. Blisnick, S. Bonnefoy, J. Jung, M. Lemos, S. Marco, S. Vaughan, S. Trépout, J.Y. Tinevez, and **P. Bastin**. 2018a. Bidirectional intraflagellar transport is restricted to two sets of microtubule doublets in the trypanosome flagellum. J Cell Biol. 217:4284-4297. *First demonstration of restricted motor movement on specific flagellar microtubules using advanced 3-D electron microscopy and high-resolution live imaging. Highlighted in F1000. Top 5% score of all articles on Altmetrics. Spotlight: Avasthi, P.* ***J. Cell Biol*** *217:4055-4056.*

3. Bertiaux, E., B. Morga, T. Blisnick, B. Rotureau, and **P. Bastin**. 2018b. A Grow-and-Lock Model for the Control of Flagellum Length in Trypanosomes. Curr Biol. 28:3802-3814 e3803. *New model to explain flagellum length control in trypanosomes.*

4. **Mallet, A., and Bastin, P**. 2022. Restriction of intraflagellar transport to some microtubule doublets: An opportunity for cilia diversification? Bioessays, e2200031.10.1002/bies.202200031. *Front cover. Highlighted by the journal: Pan, J. BioEssays, 10.1002/bies.202200082, 44, 7 (2022).*

5. Fredlund J, Santos JC, Stévenin V, Weiner A, Latour-Lambert P, Rechav K, **Mallet A**, Krijnse-Locker J, Elbaum M, Enninga J. The entry of Salmonella in a distinct tight compartment revealed at high temporal and ultrastructural resolution. Cell Microbiol. 2018 Apr;20(4). doi: 10.1111/cmi.12816. Epub 2018 Jan 23. PMID: 29250873